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L1 WITH (synthase or synthetase)	2

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DATE: Monday, August 12, 2002 Printable Copy Create Case

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	DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ		
L2	L1 WITH (synthase or synthetase)	2	L2
L1	sialyltransferase WITH (fusion adj protein)	14	L1

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L2: Entry 2 of 2 File: PGPB Jan 3, 2002

PGPUB-DOCUMENT-NUMBER: 20020001831
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020001831 A1

TITLE: Low cost manufacture of oligosaccharides

PUBLICATION-DATE: January 3, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Defrees, Shawn	North Wales	PA	US	
Johnson, Karl	Willow Grove	PA	US	

APPL-NO: 09/ 757289 [PALM]
DATE FILED: January 8, 2001

RELATED-US-APPL-DATA: *** TEST ***
Application 09/757289 is a continuation-of US application 09/442111, filed November 17, 1999, PENDING
Application is a non-provisional-of-provisional application 60/109031, filed November 18, 1998,
Application is a non-provisional-of-provisional application 60/109096, filed November 19, 1998,

INT-CL: [07] C12 P 19/26, C12 P 19/04, C08 B 37/00

US-CL-PUBLISHED: 435/101; 435/84, 536/53
US-CL-CURRENT: 435/101; 435/84, 536/53

REPRESENTATIVE-FIGURES: NONE

ABSTRACT:

This invention provides recombinant cells, reaction mixtures, and methods that are useful for the enzymatic synthesis of product saccharides. The recombinant cells contain a heterologous gene that encodes a glycosyltransferase which catalyzes at least one step of the enzymatic synthesis, as well as a system for generating a nucleotide sugar that can serve as a substrate for the glycosyltransferase.

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L2: Entry 2 of 2 File: PGPB Jan 3, 2002

09/757,289. Chris Fonda's Case. He is Junior to mine

DOCUMENT-IDENTIFIER: US 20020001831 A1
TITLE: Low cost manufacture of oligosaccharides

Brief Description of Drawings Paragraph (7):
[0015] FIGS. 6A and 6B show schematics of two examples in which two types of organisms are used to produce the nucleotide sugar. In each case, one cell type (Corynebacterium) produces a nucleotide, and the other cell type catalyzes the addition of a sugar to the nucleotide to form the nucleotide sugar. The second cell type also expresses the corresponding glycosyltransferase, which is encoded by an exogenous gene. In FIG. 6A, the desired reaction product is .alpha.-1,3-Gal-LacNAc. The reaction mixture contains Corynebacterium or yeast, for example, which naturally synthesize UTP from UDP. The UTP is activated to form UDP-galactose by the second cell type, which includes exogenous genes that encode the remaining enzymes of the GlcNAc cycle (i.e., UDP-Gal 4' epimerase, UDP-Glc pyrophosphorylase, hexokinase and phosphoglucomutase). Also present in the second cell type is an exogenous gene that encodes .alpha.1,3-Gal transferase. The UTP that is produced by Corynebacterium or yeast enters the E. coli cells and is converted by the cycle enzymes into UDP-Gal, which then serves as a donor for galactosyltransferase-medi- ated transfer to a LacNAc acceptor which is also present in the reaction mixture. This reaction releases UDP, which is recycled by passing into the Corynebacterium or yeast, where it is phosphorylated to UTP. The scheme shown in FIG. 6B is useful for producing 3'-sialyllactose. Corynebacterium or yeast is again used to produce the nucleotide required for the nucleotide sugar, with the cells being engineered to produce CTP by the introduction of an exogenous gene that encodes CMP-synthetase. The E. coli cells express enzymes that are involved in the synthesis of CMP-sialic acid from CTP. In this case, the CMP-sialic acid synthetase is expressed as a fusion protein with the 3'-sialyltransferase. GlcNAc epimerase and NeuAc aldolase enzymes are also produced. This pathway converts CTP to CMP-sialic acid, which then serves as a donor for transfer of sialic acid to the lactose acceptor moiety.

Detail Description Paragraph (133):
[0151] In some embodiments, the reaction mixture includes two or more types of recombinant cells. For example, an organism that produces a nucleotide triphosphate necessary for a cycle reaction can be combined with an organism that contains all of the remaining cycle enzymes necessary to produce the glycosidic linkage of interest (see, e.g., FIGS. 5A and 5B). Once combined, the two organisms work together to complete the cycle and produce the nucleotide sugar of interest. An illustrative example involves the combination of a bacteria such as Corynebacterium, which produces UTP, with an E. coli strain that contains one or more plasmids that encode the remaining enzymes of the GlcNAc cycle (Table 1). In FIG. 5A, the Corynebacterium strain naturally produces UTP from UDP; after the glycosyltransferase reaction, the UDP that is released by the reaction in the E. coli diffuses back into the Corynebacterium, where UTP is regenerated. The two organisms are permeabilized and the starting reagents of, for example, glucose, orotic acid, GlcNAc and lactose are added; the end product in this example is LNT-2. In FIG. 5B, the Corynebacterium does not produce sufficient CTP, so a CTP-synthetase gene is introduced into the cell which catalyzes the formation of CTP. The CTP diffuses into the E. coli cell, which contains an exogenous gene that encodes a fusion protein in which the catalytic domain of a 3'-sialyltransferase is linked to the catalytic domain for CMP-sialic acid synthetase. Also present in the E. coli cells are genes that encode GlcNAc epimerase and NeuAc aldolase. Yeast (for example, bakers yeast) can also be used to regenerate CTP from CMP using glucose, phosphate and CMP as the reagents.

Detail Description Paragraph (202):
[0218] A 100 mL culture of AD202 E. coli that expressed a fusion protein that includes the catalytic domain of .alpha.-2,3-sialyltransferase and CMP sialic acid synthetase was grown at 37.degree. C. on a shaker at 200 rpm. Expression of the fusion protein was induced with IPTG upon the culture's reaching of an OD.sub.600 equal to 0.85.

The culture was incubated at 30.degree. C. overnight. Approximately 2.0g of bacterial cell paste was harvested from this culture.

Detail Description Paragraph (207):
[0222] A strain of E. coli (EV240) genetically engineered to overexpress CMP-NAN (nanA neuS::Tn10 mutation) is transformed with plasmid DNA encoding an IPTG-inducible CMP-sialic acid synthetase/.alpha.2,3-sialyltr- ansferase fusion protein. A culture of these bacteria is grown and induced to make the fusion protein. To initiate the reaction, the cell pellet is added to a solution that contains 1% xylene, 250 mM glucose, 250 mM fructose, 25 mM lactose, 20 mM MgSO.sub.4-7H.sub.2O pH7.0, 100 mM KH.sub.2PO.sub.4 pH7, 10 mM sialic acid, catalytic amounts of CMP. The solution also contains 20% Bakers yeast (w/v). The yeast is used to produce and regenerate the nucleotide CTP used in the sialic acid cycle (fructose, glucose and CMP are used by the yeast to generate the CTP). The CMP-NAN synthetase catalytic domain of the fusion protein that is expressed by the E. coli generates CMP-NAN from the CTP and NAN, and the sialyltransferase catalytic domain then generates 3'sialyllactose.

CLAIMS:

22. The reaction mixture of claim 21, wherein the first cell type comprises exogenous genes that encode a) a fusion protein that comprises a polypeptide having 3'-sialyltransferase activity and a polypeptide that has CMP-sialic acid synthetase activity; and b) enzymes that catalyze the synthesis of sialic acid from GlcNAc; and the second cell type comprises an exogenous gene that encodes CMP-synthetase.

62. The method of claim 61, wherein the fusion protein comprises a CMP-sialic acid synthetase activity and a sialyltransferase activity.

L12 ANSWER 11 OF 14 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 3
ACCESSION NUMBER: 1998:510061 CAPLUS
DOCUMENT NUMBER: 129:255694
TITLE: The **synthesis** of sialylated oligosaccharides using a CMP-Neu5Ac **synthetase** /sialyltransferase **fusion**
AUTHOR(S): Gilbert, Michel; Bayer, Robert; Cunningham, Anna-Marie; DeFrees, Shawn; Gao, Yinghong; Watson, David C.; Young, N. Martin; Wakarchuk, Warren W.
CORPORATE SOURCE: Institute for Biological Sciences, National Research Council of Canada, Ottawa, ON, K1A 0R6, Can.
SOURCE: Nat. Biotechnol. (1998), 16(8), 769-772 (Aug 16)
CODEN: NABIF9; ISSN: 1087-0156
PUBLISHER: Nature America
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Large-scale enzymic **synthesis** of oligosaccharides, which contain terminal N-acetyl-neuraminic acid residues requires large amts. of the sialyltransferase and the corresponding **sugar-nucleotide synthetase**, which is required for the **synthesis** of the **sugar-nucleotide** donor, CMP-Neu5Ac. Using genes cloned from Neisseria meningitidis, we constructed a **fusion** protein that has both CMP-Neu5Ac **synthetase** and .alpha.-2,3-sialyltransferase activities. The **fusion** protein was produced in high yields (over 1200 U/L, measured using an .alpha.-2,3-sialyltransferase assay) in Escherichia coli and functionally pure enzyme could be obtained using a simple protocol. In small-scale enzymic **syntheses**, the **fusion** protein could sialylate various oligosaccharide acceptors (branched and linear) with N-acetyl-neuraminic acid as well as N-glycolyl- and N-propionyl-neuraminic acid in high conversion yield. The **fusion** protein was also used to produce .alpha.-2,3-sialyllactose at the 100 g scale using a **sugar nucleotide** cycle reaction, starting from lactose, sialic acid, phosphoenolpyruvate, and catalytic amts. of ATP and CMP.

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☐ 1. Document ID: US 20020068331 A1

L1: Entry 1 of 14 File: PGPB Jun 6, 2002

PGPUB-DOCUMENT-NUMBER: 20020068331
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020068331 A1

TITLE: Production of fucosylated carbohydrates by enzymatic fucosylation synthesis of sugar nucleotides; and in situ regeneration of GDP-fucose

PUBLICATION-DATE: June 6, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Wong, Chi-Huey	Rancho Santa Fe	CA	US	
Ichikawa, Yoshitaka	San Diego	CA	US	
Shen, Gwo-Jenn	Carlsbad	CA	US	
Liu, Kun-Chin	New Haven	CT	US	

US-CL-CURRENT: 435/74; 435/72

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 2. Document ID: US 20020034805 A1

L1: Entry 2 of 14 File: PGPB Mar 21, 2002

PGPUB-DOCUMENT-NUMBER: 20020034805
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020034805 A1

TITLE: FUSION PROTEINS FOR USE IN ENZYMATIC SYNTHYESIS OF OLIGOSACCHARIDES

PUBLICATION-DATE: March 21, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
GILBERT, MICHEL	HULL		CA	
YOUNG, N. MARTIN	GLOUCESTER		CA	
WAKARCHUK, WARREN W.	GLOUCESTER		CA	

US-CL-CURRENT: 435/193; 435/183, 435/200, 435/320.1, 435/325, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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☐ 3. Document ID: US 20020019342 A1

L1: Entry 3 of 14 File: PGPB Feb 14, 2002

PGPUB-DOCUMENT-NUMBER: 20020019342
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020019342 A1

TITLE: In vitro modification of glycosylation patterns of recombinant glycopeptides

PUBLICATION-DATE: February 14, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Bayer, Robert	San Diego	CA	US	

US-CL-CURRENT: 514/8; 435/14

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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☐ 4. Document ID: US 20020001831 A1

L1: Entry 4 of 14 File: PGPB Jan 3, 2002

PGPUB-DOCUMENT-NUMBER: 20020001831
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020001831 A1

TITLE: Low cost manufacture of oligosaccharides

PUBLICATION-DATE: January 3, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Defrees, Shawn	North Wales	PA	US	
Johnson, Karl	Willow Grove	PA	US	

US-CL-CURRENT: 435/101; 435/84, 536/53

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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☐ 5. Document ID: US 6319695 B1

L1: Entry 5 of 14 File: USPT

US-PAT-NO: 6319695
DOCUMENT-IDENTIFIER: US 6319695 B1

TITLE: Production of fucosylated carbohydrates by enzymatic fucosylation synthesis of sugar nucleotides; and in situ regeneration of GDP-fucose

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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☐ 6. Document ID: US 6218161 B1

L1: Entry 6 of 14 File: USPT

US-PAT-NO: 6218161
DOCUMENT-IDENTIFIER: US 6218161 B1

TITLE: Sugar-chain synthetase and process for producing the same

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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☐ 7. Document ID: US 6210933 B1

L1: Entry 7 of 14 File: USPT

US-PAT-NO: 6210933
DOCUMENT-IDENTIFIER: US 6210933 B1

TITLE: Recombinant .alpha.-2,3-sialyltransferases and their uses

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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☐ 8. Document ID: US 6096529 A

L1: Entry 8 of 14 File: USPT

US-PAT-NO: 6096529
DOCUMENT-IDENTIFIER: US 6096529 A

TITLE: Recombinant .alpha.-2,3-sialyltransferases and their uses

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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☐ 9. Document ID: US 5962294 A

L1: Entry 9 of 14 File: USPT

US-PAT-NO: 5962294
DOCUMENT-IDENTIFIER: US 5962294 A

TITLE: Compositions and methods for the identification and synthesis of sialyltransferases

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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☐ 10. Document ID: US 5858751 A

L1: Entry 10 of 14 File: USPT

US-PAT-NO: 5858751
DOCUMENT-IDENTIFIER: US 5858751 A

TITLE: Compositions and methods for producing sialyltransferases

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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☐ 11. Document ID: US 5776772 A

L1: Entry 11 of 14 File: USPT

US-PAT-NO: 5776772
DOCUMENT-IDENTIFIER: US 5776772 A

TITLE: Method for producing secretable glycosyltransferases and other golgi processing enzymes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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☐ 12. Document ID: US 5541083 A

L1: Entry 12 of 14 File: USPT

US-PAT-NO: 5541083
DOCUMENT-IDENTIFIER: US 5541083 A

TITLE: Method for producing secretable glycosyltransferases and other golgi processing enzymes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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☐ 13. Document ID: US 5032519 A

L1: Entry 13 of 14 File: USPT

US-PAT-NO: 5032519
DOCUMENT-IDENTIFIER: US 5032519 A

TITLE: Method for producing secretable glycosyltransferases and other Golgi processing enzymes

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

☐ 14. Document ID: EP 1080221 A1 FI 104266 B1 WO 9960152 A1 AU 9940433 A

L1: Entry 14 of 14 File: DWPI Mar 7, 2001

DERWENT-ACC-NO: 2000-065035
DERWENT-WEEK: 200114

TITLE: Sialylating glycoproteins efficiently

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw Desc | Image

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Terms	Documents
sialyltransferase WITH (fusion adj protein)	14

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☐ 1. Document ID: US 20020034805 A1

L2: Entry 1 of 2 File: PGPB Mar 21, 2002

PGPUB-DOCUMENT-NUMBER: 20020034805
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020034805 A1

TITLE: FUSION PROTEINS FOR USE IN ENZYMATIC SYNTHYESIS OF OLIGOSACCHARIDES

PUBLICATION-DATE: March 21, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
GILBERT, MICHEL	HULL		CA	
YOUNG, N. MARTIN	GLOUCESTER		CA	
WAKARCHUK, WARREN W.	GLOUCESTER		CA	

US-CL-CURRENT: 435/193; 435/183, 435/200, 435/320.1, 435/325, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. Desc | Image

☐ 2. Document ID: US 20020001831 A1

L2: Entry 2 of 2 File: PGPB Jan 3, 2002

PGPUB-DOCUMENT-NUMBER: 20020001831
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020001831 A1

TITLE: Low cost manufacture of oligosaccharides

PUBLICATION-DATE: January 3, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Defrees, Shawn	North Wales	PA	US	
Johnson, Karl	Willow Grove	PA	US	

US-CL-CURRENT: 435/101; 435/84, 536/53

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. Desc | Image